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Multiplex PCR for Rapid and Improved Diagnosis of Bloodstream Infections in Liver Transplant Recipients

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This prospective study evaluated the utility of the SeptiFast (SF) test in detecting 25 clinically important pathogens in 225 blood samples from 170 intensive care unit (ICU) patients with suspected sepsis after liver transplantation (LTX) or after other major abdominal surgery (non-LTX). SF yielded a significantly higher positivity rate in the LTX group (52.3%) than in the non-LTX group (30.5%; $P = 0.0009$). SF may be a powerful tool for the early diagnosis of bloodstream infections in LTX patients.

Liver transplant recipients are at high risk of developing bloodstream infections (BSIs). Bacteremia has been documented in 20% to 40% of patients after liver transplantation (12, 13, 17), with associated mortality rates ranging from 15% to 36% (17). Invasive fungal infections such as candidiasis and invasive aspergillosis have also been reported in 5% to 42% of liver transplant recipients, with associated mortality rates of 25% to 71% (4, 10, 13). A commercially available multiplex PCR assay is the SeptiFast (SF) test (Roche Diagnostics, Mannheim, Germany), which is designed to detect the DNA of 25 clinically important bacteria and fungi in the blood within 6 h. In this study, the diagnostic performance of SF was for the first time compared to that of blood culture (BC) in patients with suspected sepsis after liver transplantation (LTX) or other major abdominal surgery (non-LTX).

This prospective observational study was carried out from May 2009 to April 2011 in the Department of General, Visceral, and Transplant Surgery of the University Hospital Essen. A total of 225 blood samples for blood culture analysis and SF testing were obtained in parallel from 170 intensive care unit (ICU) patients with suspected sepsis according to the criteria of the ACCP/SCCM (7). We separated patients into groups according to surgical procedure: those who had undergone liver transplantation and those who had undergone other major abdominal surgery. Patient characteristics are presented in Table 1.

Blood was collected by venipuncture or via a central venous catheter. At least 2 sets of BC bottles (Bactec 9240 Plus Aerobic/F and Anaerobic/F; Becton Dickinson, Heidelberg, Germany) were inoculated with 8 to 10 ml of blood per bottle and incubated for up to 5 days at 36°C.

SF is a European Council (CE)-marked *in vitro* diagnostic reagent kit for the detection of DNA from bacteria and fungi in human EDTA blood. For SF testing, two tubes of 3 ml EDTA blood each were drawn from the same venipuncture or catheter at the same time as the BCs were obtained. A detailed description of the SF workflow has been previously published (6). The spectrum of species that can be detected by SF is shown in Table 2. According to a publication by Richter et al., pathogens from skin flora were considered to be probable contaminants when detected in only one of two or more BCs (11). Categorical variables were compared with the chi-square test. Statistical analysis was performed with SAS version 9.2.

TABLE 1 Baseline demographic characteristics of 170 patients with suspected sepsis

Characteristic	No. (%) of patients with:[a]	
	LTX group ($n = 79$ patients)	Non-LTX group ($n = 91$ patients)
Mean age ± SD; range (yr)	52.6 ± 10.9; 27–70	60.2 ± 13.2; 28–88
Female	28 (35.4)	46 (50.5)
Cause of cirrhosis		
Alcoholic	21 (26.5)	
Infectious (hepatitis B/C)	17 (21.5)	
NASH	4 (5.0)	
Other (autoimmune, unknown)	11 (13.9)	
Hepatocellular carcinoma	12 (15.1)	
Primary sclerosing cholangitis	7 (8.8)	
Acute liver failure	4 (5.0)	
Liver cysts	3 (3.7)	
Malignancy		48 (60.7)
Abdominal infection		27 (29.6)
Abdominal organ perforation		12 (13.1)
Colon ischemia		4 (4.3)

[a] Except for the first row (mean age), values are numbers (percentages) of patients. LTX, patients after liver transplantation; non-LTX, patients after other major abdominal surgery; NASH, nonalcoholic steatohepatitis.

The overall positivity rate for the entire cohort was 40.8% by SF and 35.5% by BC ($P = 0.11$). SF and BC yielded concordant negative results in 110 (48.9%) samples and concordant positive results in 57 (25.3%) (Table 3). The results from 35 (15.6%) paired blood samples were SF^+/BC^-, whereas the results from 23 (14.2%) samples were SF^-/BC^+. In 10 of these 23 SF^-/BC^+ samples, the microorganisms were considered to be contaminants.

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TABLE 2 Spectrum of species that can be detected by SeptiFast

Type of organism	Organism
Gram positive	*Staphylococcus aureus*
	Coagulase-negative staphylococci
	Streptococcus pneumoniae
	Streptococcus spp.
	Enterococcus faecium
	Enterococcus faecalis
Gram negative	*Escherichia coli*
	Klebsiella (pneumoniae/oxytoca)
	Serratia marcescens
	Enterobacter (cloacae/aerogenes)
	Proteus mirabilis
	Pseudomonas aeruginosa
	Acinetobacter baumannii
	Stenotrophomonas maltophilia
Fungus	*Candida albicans*
	Candida tropicalis
	Candida parapsilosis
	Candida krusei
	Candida glabrata
	Aspergillus fumigatus

The positivity rate for SF was significantly higher in the LTX group (52.3%) than in the non-LTX group (30.5%; $P = 0.0009$). BC also yielded more positive results in the LTX group (43.9%) than in the non-LTX group (27.9%; $P = 0.013$). Sensitivity, specificity, and positive and negative predictive values for both groups are presented in Table 3. More than 50% of the SF^-/BC^+ samples were coagulase-negative staphylococci. Among those bacteria that SF detected in addition to those isolated by BC, *Enterococcus* spp. and *Enterobacteriaceae* were the most frequently identified. In 7 specimens (10.8%), *Aspergillus fumigatus* was detected only by SF (Table 4).

The performance of SF has been evaluated in a variety of patient populations, but information about its performance in transplant patients is scarce. Our results are in agreement with previous reports which also showed that the rates of positive findings are higher for SF than for BC in patients with febrile neutropenia, in newborns and children, and in septic ICU patients (5, 8, 9, 16, 18, 19).

TABLE 3 Diagnostic performance of blood culture and SeptiFast in 225 samples from patients with suspected sepsis

Result[a]	Value[c]		
	Entire cohort ($n = 225$)	LTX ($n = 107$)	Non-LTX ($n = 118$)
BC^+	80 (35.5)	47 (43.9)	33 (27.9)
SF^+	92 (40.8)	56 (52.3)	36 (30.5)
SF^+/BC^+	57 (25.3)	38 (35.5)	19 (16.1)
SF^-/BC^-	110 (48.9)	42 (39.3)	68 (57.6)
SF^+/BC^-	35 (15.6)	18 (16.8)	17 (14.4)
SF^-/BC^+	23 (10.2)	9 (8.4)	14 (11.8)
Sensitivity[b] of SF (%)	71.2	80.9	57.6
Specificity[b] of SF (%)	75.9	70.0	80.0
Positive predictive value of SF (%)	62.0	67.9	52.8
Negative predictive value of SF (%)	82.7	82.3	82.9

[a] SF, SeptiFast; BC, blood culture.
[b] Considered BC as gold standard.
[c] Values are the numbers (percentages) of samples unless otherwise noted. LTX, patients after liver transplantation; non-LTX, patients after major abdominal surgery.

TABLE 4 Spectrum of pathogens detected by blood culture, SeptiFast, or both in 225 samples from patients with suspected sepsis

Microorganism	No. (%) of isolates[a]		
	SF^+/BC^+	SF^+/BC^-	SF^-/BC^+
Gram-positive cocci	21 (34.4)	21 (32.3)	16 (61.5)
Staphylococcus aureus	5 (8.2)	4 (6.2)	1 (3.8)
Coagulase-negative staphylococci	5 (8.2)	3 (4.6)	14 (53.8)
Enterococcus spp.	11 (18.0)	14 (21.5)	1 (3.8)
Gram-negative rods	34 (55.7)	30 (46.2)	5 (19.2)
Enterobacteriaceae	25 (41.0)	22 (33.8)	4 (15.4)
Pseudomonas aeruginosa	8 (13.1)	6 (9.2)	1 (3.8)
Stenotrophomonas maltophilia	1 (1.6)	2 (3.1)	0 (0.0)
Fungi	5 (9.8)	11 (16.9)	3 (11.5)
Candida spp.	6 (9.8)	4 (6.2)	3 (11.5)
Aspergillus fumigatus	0 (0.0)	7 (10.8)	0 (0.0)
Other[b]	0 (0.0)	3 (4.6)	2 (7.7)
Total[c]	61 (100)	65 (100)	26 (100)

[a] SF, SeptiFast; BC, blood culture.
[b] The other pathogens detected in SF^+/BC^- samples included *Acinetobacter baumannii* (detected once) and *Streptococcus* spp. (detected twice), and the other pathogens detected in SF^-/BC^+ samples included *Aeromonas hydrophila* and *Cryptococcus neoformans*.
[c] Number of samples in which more than one organism was detected: SF^+/BC^+, 4; SF^+/BC^-, 30; SF^-/BC^+, 3.

The rapid availability of the results of SF may influence early therapeutic decisions for septic patients, particularly when potentially more-resistant pathogens are detected. The presence of these pathogens, which are not always covered by initial, empirical antibiotic therapy, is an important cause of treatment failure in septic patients. Another reason for using SF to analyze blood samples from critically ill patients such as LTX patients is that the detection of yeast and filamentous fungi in blood is limited to their specific growth abilities. Invasive aspergillosis (IA) occurs in 1% to 10% of LTX patients (2, 14), with a high mortality rate (15). SF detected *A. fumigatus* in blood from 4 LTX and 3 non-LTX patients. According to the revised EORTC/MSG criteria (3), 5 of these 7 patients had probable IA and 2 had proven IA. In neutropenic patients, two studies highlighted the value of SF in the rapid diagnosis of IA. When data from these studies are taken together, SF detected DNA from *A. fumigatus* in all 9 patients with probable IA, whereas the results of BC were negative (9, 16).

In contrast to all previous performed studies, the present study found that the overall rate of positive results by SF was very high (52.3%) in the LTX group, even though a high percentage of the patients included in the study had received empirical or preemptive antimicrobial therapy. This might lead to the hypothesis that in septic LTX patients, the bacterial load is higher than in the non-LTX group. Therefore, LTX patients with suspected sepsis seem to be a good cohort for the use of SF for rapid detection of pathogens in the bloodstream.

One limitation of the present study is that we did not attempt

to correlate the SF^+/BC^- results with anti-infective treatment, the response to a specific therapy, or clinical outcome. However, findings of a multicenter trial comparing BC with SF in severe human sepsis showed that pathogen detection by SF was positively correlated with biomarkers of host response and disease severity even if the results of BC were negative (1).

SF was more sensitive than BC in LTX patients, a finding suggesting that LTX patients with suspected sepsis are a good cohort for the rapid and improved diagnosis of bloodstream infections by SF.

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